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MICROBIOLOGICAL CRITERIA FOR AEROSPACE POTABLE WATER SYSTEMS

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FOREWORD

This work was performed by the Biospecialties Branch, Physiology Division of the Biomedical Laboratory in support of project 7164, Biomedical Criteria for Aerospace Flight, task 716410, Aerospace Sanitation and Personal Hygiene. The work was conducted from February 1964 to March 1965. Dr. Arnold R. Slonim was task engineer. Mr. Courtney Metzger, Chief Requirements and Evaluation Branch, and Mr. Albert B. Hearld supplied the processed water obtained from various recovery devices.

This technical report has been reviewed and is approved.

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ABSTRACT

Extended space missions will necessitate that life support subsystems operate on a closed ecological or bioregenerating principle. This entails a continual recycling or regeneration of materials to sustain space crews. Potable water may be made available by a variety of techniques from various sources, such as urine and atmospheric condensate. Microbiological potability standards that can realistically be applied to space water supplies are dictated by the inherent technological problems and logistic limitations. With these in mind, a standard of sterility is proposed and the reasons for this stringent requirement are indicated. For ground based studies, a less stringent standard is also compatible with equipment capabilities and experimental requirements.

MICROBIOLOGICAL CRITERIA FOR AEROSPACE POTABLE WATER SYSTEMS*

As the duration of space missions increases, the requirement for a reliable means to reclaim water becomes more pressing. The logistics of contemplated space systems necessitate that life support equipment approach an idealized bioregenerating or closed ecological system. Design criteria for this equipment must be based upon sound biological, chemical, and engineering principles. The purpose of this report is to present some tentative biological standards and the philosophy upon which they are based, as well as some microbiological data obtained from water samples resulting from various urine and other waste water recovery devices. The performance of these devices is compared to the microbiological standard for aerospace water proposed in this report. The standard--that of absolute sterility--is recognized as being extremely stringent. The reasons for its choice are presented below:

The current Public Health Standards (ref 2) are not concerned with the identification of all organisms contained in a water source nor in the total number present. Rather, these microbiological criteria serve to indicate the presence of fecal contamination, this contamination having recently occurred. The standards are based on the recovery of the characteristic residents of the animal intestinal tract--the coliform group and more speifically, Escherichia coli. Routine procedures are available for the detection of these fecal bacteria (ref 1, 3). Some water bacteriologists recommend the use of enterococci as a more reliable indication of fecal pollution. British water standards are based on the isolation of yet another intestinal inhabitant, Clostridium perfringens (welchii) since it remains viable longer than the coliform organism and hence is indicative of past pollution. Of major significance is that only these organisms are used to validate the potability of water supplies. The condemnation of a given water source is based not on absolute numbers recovered, but rather on the statistical recovery of the indicator organism from a particular number of samples tested over a given period. The application of this probability concept is exemplified by Kehr and Butterfield (ref 5) who analyzed probable infection rates of bathers based upon coliform: Salmonella typhosa ratios in sewage polluted water. Thus, the philosophy of determining the microbial potability is based on an indication of fecal contamination and not on an intensive microbiological analysis.

Although the routine analytical methods described in the literature are rather simple, they are quite time consuming and require a considerable amount of microbiological equipment. The constraints imposed upon space systems (i.e., weight volume, and power) and the inherently poor microbiological quality of water to be reclaimed invalidate the use of these standards. Since extended space missions will impose personal hygiene problems, more stringent methods must be applied to eliminate the hazards inherent in restricted environments. The additional stresses of space flight may have significant effects on the physiological status of crew members and, in particular, may influence the ecological interrelationships of man and his indigenous microflora.

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*A condensed version of this report is presented in Slonim, A. R., A. Roth, A. Hearld, S. A. London and A. West 1967 Potable Water Standards for Aerospace Systems-1967, AMRL-TR-67-25, Wright-Patterson AFB, Ohio.

The many water reclamation systems and sources (i.e., urine, atmospheric condensation, wash water, fuel cell water, and , perhaps, feces) being considered for long duration space systems (ref 8, 9) are extremely different from terrestial water sources. The most obvious difference is that the waste water must be reused and purified continuously. If the entire water cycle on earth is compared to that required in a closed space system, the difference is really quantitative, not qualitative. In other words, both operate on a cyclical basis and both must reprocess waste water. The space system differs because of the proximity of waste water to pure water and its logistical limitations. Thus, a space water purification process must accomplish all that its immensely larger counterpart does, but must do so with considerably more efficiency and reliability.

Although earth-bound water purification processes can no longer be satisfied with rather haphazard approaches, since waste disposal can no longer resort to dilution as an easy answer, the space system water purification inherently deals with a more complex problem. Its processing must handle essentially sewage, thus the chemical and biologic burden imposed is a difficult technilogic challenge.

Attendant with this problem, from which there is no recourse, are the logistic limitations imposed on the space system. Though the purification of the water becomes an exercise in engineering efficiency, the measurement of the quality of the processed product is the major obstacle. Standards for such water could be readily established, based upon many years of public health experience. However, the means by which these standards can be measured and monitored under the restricted environment of a space system are the limiting factors. The judicious choice of chemical standards and the selection of appropriate physicochemical monitoring techniques can be achieved. Considerations of the restricted population involved and the relaxation of the commitment to lifetime consumption of the water can ease space water chemical standards, at least with respect to current USPHS standards. In addition, knowledge of the chemical substances that are potential contaminants (perhaps less diverse that found in terrestrial water sources) may mollify harsher demands.

This is not so with the biological contaminants that could gain entry into the water supply. All of the sources of waste water mentioned previously harbor various kinds and numbers of microbes and, in addition, provide nutrients for the further growth of contaminating organisms. These added contaminants arise from dirt within the system as well as those shed by the space crew. Preliminary studies have shown atmospheric condensate obtained during studies conducted in the Aerospace Medical Research Laboratories Life Support Systems Evaluator is grossly contaminated. This condensate collector used as part of the environmental control system serves not only as a humidity control device, but also acts as an efficient scrubber of many types of organic, inorganic, soluble, and particulate material present in the recirculated air. The airborne microflora, provided by skin shedding, fomites, food residues, and indigenously, find the condensate a habitable environment. Although mid-stream samples of urine from males are sterile, they do not remain so for long, since urine can be a reasonably good culture medium. Fuel cell water also contains considerable numbers of microbial contaminants (ref 7). Thus, a significant quantity and diversity of microorganisms must be suspect as potential contaminants of the potable water supply and standards must be established.

If it were possible to obtain a device that could (1) sample and concentrate the water supply; (2) perform a detailed qualitative, differential determination of the microbes present (viruses, PPLO, bacteria, etc.) and (3) provide this information in some readily useable form, such as a tape print out, within 30 minutes, standards for space water supplies could be almost self-engendering. Such a device is a feat of the distant future. The determinative procedures used in microbiological laboratories today require several days of manipulatory and incubation time, even if certain newer methods are employed. Approaches such as gas chromatography (ref 4) applied to the volatile products of microbial metabolism still require controlled conditions and computer analyses. This method applies to bacteria only; fungal identification requires more extensive incubation times and primarily morphologic criteria, while viral identification entails even longer incubation periods, complex tissue culture, and immunologic methods. Thus, the selection of biologic water standards must be restricted to bacterial species that are indicative of contamination of human origin. The question then resolves itself to "What particular members of the human bacterial microflora are amenable to rapid diagnostic identification?" Unfortunately, the answer for space systems is none of them. This is based not on the absence of likely candidates, but rather on the absence of appropriate detection techniques and devices. Therefore, a standard of "sterile" must be imposed upon space water supplies by virtue of the system itself. With such a standard, continual monitoring techniques are available, such as electronic particle counting and enzymatic methods (e.g., the luciferin-luciferinase reaction, using ATP from contaminating microbes, ref 6). The latter method may be capable of detecting one organism per milliliter; with adequate concentration procedures, such as membrane filtration, this may be increased to encompass the entire water reservoir.

This standard of "sterility" would at first appear too stringent and perhaps beyond the current state-of-the-art. Examination of some data derived from several water reclamation devices investigated in our Laboratories (see table I) is in order at this time to warrant the application of this rigid standard. Devices based on several different principles have been investigated in the Aerospace Medical Research Laboratories. These include the following:

Compression Distillation, Absorption Filtration (CDAF)

This approach uses the processes of compression distillation and absorption filtration in a low pressure, semiautomatic device designed to operate either in a weightless state or with gravity acting towards the mounting base.

Electro-process (EP)

This recovery system consists of two platinum electrodes passing 6 amps through the product, (urine, waste water, etc.) that is agitated by a stirrer. The urea is decomposed and evolves as ammonia. The solids (salts), are removed by reverse osmosis or electrodialysis.

Ultrafiltration (UF) (Reverse Osmosis)

In the process of osmosis, water flows from the area of low salt concentration through a membrane to the area of higher salt concentration. This condition creates osmotic pressure. The principle of ultrafiltration imposes a pressure on the concentrated salt solution that is greater than the osmotic pressure and reverses the flow, thus, reverse osmosis. By using selected membranes, the solution can be desalted. A pressure of 50 atmospheres (750 psi) is needed to deliver a reasonable flow through these membranes.

Membrane Permeation (MP)

This process produces potable water by using a temperature gradient to make a single passage of liquid through selective membranes.

Thermoelectric (TE)

Thermoelectric modules have the ability to transfer heat across areas having small temperature differences. This eliminates the need for large heat transfer surfaces and, in turn, reduces the size, power, and weight of the recovery unit.

Electrodialysis (ED)

Some plastic membranes have the property of passing only electrically positive particles (cations) and others, of passing only electrically negative particles (anions). The salts in urine break up into equal parts of cations and anions and move in opposite directions in an electrical field. An electrodialysis cell consists of alternate cation- and anion-permeable membranes.

Atmospheric Condensate (AC)

Moisture in the recirculated air within the Life Support System Evaluator was removed by condensation on cooling coils. The condensate was removed daily from the Evaluator and subjected to passage through various filters, singly or in combination, such as charcoal and bacterial filters. Certain collections included the use of chemical disinfectants. For approximately one year, the reclaimed water was subjected to cursory bacteriological analysis, by plating diluted and undiluted samples on Trypticase Soy Agar (BBL) and incubating at 37 C for 48 hours. Table I shows the results of these preliminary studies. Bacterial contamination occurred in all experimental devices to a greater or lesser extent and, most notable, the devices (including atmospheric condensate) were quite unreliable as to the degree or frequency of contamination. The design principles of several of these devices suggest better quality water (microbially) should be produced. Experience has indicated the contrary. The microbes isolated during these studies represented many bacterial genera. Since we cannot distinguish a pathogen from a harmless microbe under space system conditions, and since the absence of bacterial forms should insure the absence of other pathogenic entities indigenous to man (viruses, etc.) only a water supply devoid of any bacterial forms can insure the potability of the supply

TABLE I

BACTERIA RECOVERED FROM RECLAIMED WATER

Sample Date	*Source or Process*[1]	*Bacteria/ml*
2-20-64	Raw Urine	20.5×10^5
2-20-64	CDAF	18×10^5
2-20-64	CDAF + PF	615
2-21-64	CDAF + PF	6×10^3
2-24-64	CDAF + PF	57×10^3
2-25-64	CDAF + PF	2×10^5
3-2-64	CDAF + PF[2]	275
3-3-64	CDAF + PF	Negative
3-4-64	CDAF + PF	Negative
3-11-64	CDAF + PF[3]	115
3-12-64	CDAF + PF	1.6×10^5
3-13-64	CDAF + PF	2.5×10^5
3-17-64	CDAF + PF	6.5×10^5
3-16-64	EP	Negative
3-17-64	EP	Negative
3-18-64	EP + PF	4.5×10^3
3-19-64	Raw Urine	$> 10^6$
3-19-64	EP	Negative
3-23-64	EP	64×10^3
4-3-64	EP + PF	Negative
4-7-64	EP + PF	Negative
4-14-64	Raw Urine	12×10^3
4-14-64	EP + PF	Negative
4-14-64	EP + CDAF	Negative
4-14-64	EP + CDAF + PF	Negative
4-15-64	EP + PF	Negative
4-16-64	Raw Urine	6×10^3
4-16-64	EP + PF	Negative
4-17-64	EP + PF	Negative
4-20-64	EP + CDAF	22×10^3
4-20-64	EP + CDAF + PF	Negative
4-23-64	EP	28×10^7
4-23-64	EP + PF	106×10^3
4-28-64	EP	103×10^5
4-29-64	EP	29×10^5
4-30-64	EP	27×10^5
5-1-64	EP	2×10^5
5-1-64	EP + PF	Negative
5-5-64	EP	31×10^5
5-5-64	EP + PF	Negative
5-6-64	EP	123×10^5
5-7-64	EP	36×10^5
5-7-64	EP + PF	Negative
5-8-64	EP	7×10^5
5-8-64	EP + PF	780
5-12-64	EP	131×10^3
5-12-64	EP + PF	20
5-13-64	EP	39×10^5
5-13-64	EP + PF	Negative
6-10-64	MP	7×10^3
7-21-64	MP	24×10^3
6-10-64	UF	$> 10^7$
6-26-64	UF	26×10^3
6-30-64	UF	15×10^6
7-1-64	UF	100
7-13-64	UF	8×10^3
7-22-64	UF	31×10^5
2-19-65	UF	32×10^5
2-19-65	UF	Negative
8-15-64	TE + PF	150×10^3
10-23-64	TE + PF	Negative
10-26-64	TE + PF	58×10^5
10-27-64	TE	44×10^5
10-28-64	TE	91×10^5
10-29-64	TE + PF	103×10^5
2-9-65	TE + PF	160
2-10-65	TE + PF	70
12-15-64	ED + PF	Negative
12-21-64	ED + PF	15×10^3
12-22-64	ED + PF	Negative
12-23-64	ED + PF	50×10^3
12-23-64	ED + PF	Negative
12-24-64	ED + PF	30×10^3
1-6-65	ED + PF	Negative
1-13-65	ED + PF	12×10^3
1-14-65	ED + PF	Negative
1-20-65	ED + PF	33×10^3
1-21-65	ED + PF	84×10^3
1-22-65	ED + PF	Negative
1-26-65	ED + PF	Negative
1-27-65	ED + PF	22×10^5
1-29-65	ED + PF	16×10^3
2-2-65	ED + PF	12×10^3
2-5-65	ED + PF	31×10^5
2-6-65	ED + PF	9×10^5
2-8-65	ED + PF	Negative
2-10-65	ED + PF	32×10^5
2-10-65	ED + PF	21×10^5
2-11-65	ED + PF	29×10^5
2-12-65	ED + PF	29×10^5
2-18-65	ED + PF	12×10^5
2-18-65	ED + PF	8×10^5
2-24-65	ED + PF	120
2-26-65	ED + PF	27×10^3
3-1-65	ED + PF	80×10^3
10-23-64 to 10-28-64	AC	Average: 40×10^3 (4 Samples)
7-25-64 to 3-3-65	AC + PF	13 Samples Negative; 35 Samples Range 30 to $> 10^7$

[1] CDAF = Compression Distillation-Absorption Filtration
PF = Pall Ultipor® Filter
EP = Electro Process
MP = Membrane Permeation
UF = Ultra Filtration
TE = Thermo Electric
ED = Electro Dialysis
AC = Atmospheric Condensate, Untreated

[2] Washed with Roccal®, Pall Filter autoclaved

[3] Sterilized with Cryoxide for 5.5 hours and purged with air

(assuming chemical acceptability also). Application of this standard to a water supply under actual space flight conditions then becomes feasible. The detection of any viable bacteria (the number depending upon the sensitivity of the method) indicates a deterioration in the processing unit.

We are of the opinion that devices able to meet these rigid requirements are within the capability of present day technology. The use of redundancy in the system can accomplish this without extensive logistic penalties. The designer of a water reclamation system must first consider a technique to preserve the waste water source (i.e., prevent further microbial activity). This may be done by adding disinfectants commensurate with system requirements or immediate initiation of the recovery process. The process itself, no matter what physicochemical principle it is based upon, when implemented by sound engineering and guided by biological consideration, will yield relatively clean water to meet the demands of the chemical standards. The redundancy in the system could then consist of absolute filters (various compositions and designs are available) at various points, transfer lines coated with silver (sufficient toxicologic and chemical data have been determined), temperature elevation (such as in flash pasteurization), and addition of appropriate disinfectant in the reservoir. Certainly the mission duration, crew size, and available space, weight, and power will dictate the specific design characteristics. However, for any mission that requires reprocessing of waste water, a combination of the above can be selected which is compatible with the mission logistics.

Most discussions concerning microbiological water standards dwell on the identification of the viable microbes selected as pollution indicators. Such considerations for terrestrial water supplies are certainly warranted and adequate. However, the toxicity of certain components of particular bacterial cells, as well as overt exotoxins (those liberated into the suspending milieu), requires the prevention of any microbial growth at any point within the water reclamation system. The toxic moieties of many enteric microbes (associated with the somatic or 0 antigens) are indeed a potential problem and, since their physiologic effect requires rather high concentrations, the numbers of cells possessing these substances must not be permitted to increase above that initially present in the water source. Therefore, as has been mentioned previously, immediate processing of the water or use of suitable disinfection procedures, or both, is necessary.

Finally, for most reclamation systems, the presence of residue material presents an additional hazard. Residues may be present in distillation chambers, filters, or dialyzing units and represent sources of contamination to the system. Methods must be devised to either remove this material periodically or replace parts of the unit. Again the design of the reclamation system and the logistic burden will dictate which handling approaches are indicated.

The standard of sterility has been discussed with many space system engineers and microbiologists and has received reasonable acceptance. However, there is a need for a less rigorous standard for use in ground-based closed system experiments, in view of the difficulties encountered in producing sterile water to date. Ground-based studies are not hampered by lack of time and equipment restrictions. The

subjects participating in the experiment may be given a potable water supply at the initiation of the study and the reclaimed water produced subsequently may be subjected to microbial and chemical analyses outside the test chamber. Upon verification of the potability of a particular batch of water, it may be consumed while the next batch undergoes analyses. Microbial water standards for ground-based studies are in table II. The bacteria selected as markers were chosen because they are indigenous to man and are readily identifiable by simple bacteriologic techniques. Fungi are included in the group, since their presence is indicative of generally dirty conditions in the reclamation system. Only aerobic organisms have been considered. Anaerobes have not been considered because they will provide no additional information. Assigning 200 as the maximal number of aerobes (other than those indicated in table II) is admittedly arbitrary. In our experience, water recovery devices are capable of this level of performance, if properly operated, and water of this quality can be produced over long periods of time. The absence of the normal bacterial residents of man listed in table II should insure that the water is free of other pathogens. The authors believe that such requirements are not only reasonable, but are necessary to protect human subjects when water supplies are derived from waste water sources.

TABLE II

Microbial Water Standards for Ground Based Studies[1]

Organism	Plating Medium	Maximal Allowable No.
Staphylococci	Blood Agar	0
Streptococci	Blood Agar	0
Enterococci	Azide Agar	0
E. Coli	EMB or Endo Agar	0
Pseudomonads	Sellers Agar	0
Fungi	Rose Bengal or Sabouraud Agar	0
Total Aerobes	Trypticase Soy Agar	200/ml

[1]Analyses are conducted on 100 ml aliquots of product water using membrane filtration techniques.

The final selection of a water reclamation system for space use must be based on the consistent production of sterile water. Detailed ground studies are mandatory and should eventually include viral analysis. The importance of a safe water supply demands that the reliability of the recovery system be as high as any other subsystem comprising the entire space system.

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13. ABSTRACT

Extended space missions will necessitate that life support subsystems operate on a closed ecological or bioregenerating principle. This entails a continual recycling or regeneration of materials to sustain space crews. Potable water may be made available by a variety of techniques from various sources, such as urine and atmospheric condensate. Microbiological potability standards that can realistically be applied to space water supplies are dictated by the inherent technological problems and logistic limitations. With these in mind, a standard of sterility is proposed and the reasons for this stringent requirement are indicated. For ground based studies, a less stringent standard is also compatible with equipment capabilities and experimental requirements.

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